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INSECTICIDE-RESISTANCE IN BED-BUGS

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SYNOPSIS

A simple method of measuring insecticide-resistance in bed-bugs is described. Batches of adult bugs are put on small pieces of filter-paper impregnated with oil solutions of insecticides, and mortality is estimated after 5 days at 25°C.

In both *Cimex lectularius* and *C. hemipterus*, two kinds of resistance can develop, either independently or in conjunction. One involves DDT, methoxychlor and analogues; it cannot be overcome by the addition of DMC. The other involves γ-BHC, dieldrin and various other chlorinated cyclodiene insecticides. The resistance spectrum of bed-bugs towards this group of compounds resembles those of *Anopheles gambiae* and *Musca domestica*, indicating similarity in the defence mechanism.

The use of DDT to control bed-bugs in the years following the Second World War has produced a very great reduction in infestation by this pest both in Europe and in North America (Busvine, 1957). In the tropics, DDT also proved very effective for bed-bug control; but in recent years there have been many scattered reports of resistance, mainly from the warmer climates. An early observation of failure to control bed-bugs by DDT was made in Hawaii in 1948 (Johnson & Hill). Later, reports of resistance came from Israel (Levinson, 1953), Iran,[1] and French Guiana (Floch, 1955). Similar troubles were experienced with the tropical bed-bug, *Cimex hemipterus*, which has been surviving DDT treatment in Taiwan (Chen et al., 1956), Bombay State (Rao & Halgeri, 1956), Hong Kong and Singapore (Busvine, 1957) and, more recently, the Gambia and Somaliland (see below). Apart from DDT-resistance, there have been confirmed cases of resistance of *C. lectularius* to BHC in Israel (Cwilich & Mer, 1957) and of *C. hemipterus* to dieldrin in Tanganyika [2] and the Ivory Coast (Hamon et al., 1957). Multiple resistance to all chlorinated residual insecticides does not seem to have been demonstrated; indeed the DDT-resistant bugs in Hong Kong, Taiwan and Iran have been found easily killed by BHC, while the dieldrin-resistant bugs in Tanganyika and the Ivory Coast were

[1] C. Mofidi & B. Samimi in a report submitted in 1956 to the Institute of Parasitology and Malariology, Teheran.

[2] A. Smith in a report submitted in 1957 to the East African Institute of Malaria and Vector-borne Diseases.

almost normally susceptible to DDT. Nevertheless, there are already several places where bed-bugs have developed combined resistance, as will be demonstrated below.

Methods of Detecting and Measuring Resistance

Nearly all the reports quoted were based on field observations or on rather crude laboratory tests which did not give any information on the level of resistance or on possible cross-resistance to other insecticides. One exception was the investigation in Israel (Cwilich & Mer), in which a reliable laboratory spraying apparatus was used. However, while this technique is satisfactory in a well-equipped laboratory, it is clearly unsuitable for general use everywhere, especially under field conditions. In view of the widespread nature of the problem, it seems very desirable to adopt a simple but dependable test method, which can be used by workers in different countries to provide comparable results.

One possible method would be to employ filter papers impregnated with standard oil solutions of insecticide, as described by Busvine & Nash (1953). Papers impregnated in an equivalent manner are now incorporated in the kit provided by the World Health Organization for the detection and measurement of resistance in adult mosquitos. Many of these impregnated papers are already distributed about the world and it would clearly be advantageous if they could also be employed for testing resistance in other insects, such as bed-bugs. As a very simple trial method, small pieces (about 2 × 5 cm) were cut from such treated papers, folded once and dropped into test-tubes. Batches of about 10 adult bed-bugs were added to each tube and it was found that the insects clung to the treated papers. Sometimes the bugs became paralysed by the insecticide and fell to the bottom of the tubes; in such cases, they very seldom recovered.

Preliminary tests were made with different exposures at various intervals after feeding. After exposure on the treated paper, the bugs were removed to clean papers in fresh tubes and examined for mortality a week after the start of the test. Results of these preliminary tests (based on about 20 to 30 bugs for each point) are shown in Table 1.

The figures for the dieldrin tests were plotted on log probability paper and the LC_{50} values estimated graphically. The relation between these values and the exposure period were roughly hyperbolic, but were best fitted by an expression of the form $c.t^n = K$. By plotting log c against log t, an approximate value for n was found to be 0.87; this gave fair agreement with the observed results:

Exposure time (hours):	1	2	24	48	120
LC_{50} (%) observed:	5	2.2	0.32	0.21	0.08
LC_{50} (%) calculated:	5.0	2.75	0.32	0.17	0.078

Since the value *n* is less than unity, longer exposures demand higher concentrations than would be expected if the LC_{50} were the simplest function of exposure ($c.t$ = K). Possibly this is due to some elimination of poison, which may occur at low doses, with long exposures, even in normal insects.

TABLE 1. PERCENTAGE KILLS OF ADULT BED-BUG (*CIMEX LECTULARIUS*) BY PAPERS IMPREGNATED WITH INSECTICIDE

Insecticide \ Days starved before testing	Exposure (hours)						
	1		2	24		48	120
	7-14	1	2	7-14	1	2	5
DDT							
4 %	6	0	15	65	44	63	100
2 %	—	—	—	58	21	31	79
1 %	—	—	—	6	—	—	37
Dieldrin							
4 %	43	35	89				
1.6 %	0	0	27				
0.8 %				100	100		
0.4 %				69	77	94	
0.2 %				0	18	43	100
0.1 %						0	83
0.075 %							51
0.05 %							21

With regard to the DDT tests, it was found that even normal bugs have low susceptibility to DDT immediately after feeding. After about a week, they become easier to kill with DDT, though this effect was not noted with dieldrin. Eventually it was decided to test the bugs 5 days after feeding and to give them 5 days' (120 hours') exposure. For convenience, the mortality count is made at this point, since it has apparently reached stability. Control mortality in healthy young adults of *C. lectularius* was below 5 %; with *C. hemipterus* it was always below 10 %.

Susceptibility Levels of Normal *C. lectularius*

In tests with young adult bugs, the results shown in Table 2 were obtained (15-30 bugs per batch). It will be noted that the females are distinctly more susceptible, both to DDT and to dieldrin, than the males. However, for convenience, both sexes were used in subsequent tests in roughly equal

TABLE 2. RESULTS OF PRELIMINARY TESTS TO DETERMINE SUSCEPTIBILITY LEVELS OF ADULT *CIMEX LECTULARIUS*

Insecticide concentration	Percentage kill of males	Percentage kill of females
DDT		
3%	96	100
2%	72	92
1%	15	44
0.5%	0	0
Dieldrin		
0.2%	94	100
0.1%	47	77
0.075%	6	66
0.050%	22	36

numbers, at the cost of a slight loss of precision. Occasionally, a few well-grown nymphs were included, but they were not used in computing the results. They were observed to be rather less susceptible than the adults.

Comparison of the Method adopted with Spray Tests

As a matter of interest some comparative measurements of relative resistance were made with the method described above and also by the spray tower used by Cwilich & Mer. For these spraying tests, the bugs were put into small dishes lined with clean filter-paper. The insecticides were dissolved in Risella oil and sprayed on to the bugs at the rate of 0.3 mg per cm^2, by the spray tower described by Potter (1941). Bugs were removed from the dishes on the day after spraying and kept in clean tubes for a week before mortality counts.

The two bug colonies used were: (*a*) the normal laboratory colony of *Cimex lectularius;* (*b*) a DDT-resistant colony of *C. hemipterus*. Experiments were done at various concentrations and LC_{50} values estimated by the two methods as shown in Table 3. It will be seen that the two methods present a very similar comparison of these two strains.

Strains of Bed-bug Tested

All the colonies mentioned below were started from a few dozen adults collected in the field. They are now maintained in 5 × 5 cm glass tubes covered with gauze and kept in an incubator at 25°C. Once a week they are

TABLE 3. COMPARISON OF TWO STRAINS OF BED-BUG BY A SPRAY METHOD AND BY THE IMPREGNATED FILTER-PAPER METHOD

Insecticide	Spray method			Filter-paper method		
	LC_{50}		ratio	LC_{50}		ratio
	(1) *hemipterus*	(2) *lectularius*	$\frac{(1)}{(2)}$	(1) *hemipterus*	(2) *lectularius*	$\frac{(1)}{(2)}$
DDT	3.9	1.4	2.8	3.8	1.3	2.9
BHC	0.038	0.10	0.38	0.012	0.04	0.30
Pyrethrins	0.055	0.04	1.4	0.085	0.045	1.9
Lethane 384	3.0	2.9	1.03	0.7	0.7	1.0

fed by clipping the tubes, gauze side down, on the ears of a lop-eared rabbit. The resistant strains are maintained, as received, without selective exposures to insecticide.

Cimex lectularius

Normal laboratory colony. This was originally obtained from an infestation in London in 1937 and has been maintained in laboratory culture since at the London School of Hygiene and Tropical Medicine.

Israel strain. This was kindly sent to me in April 1957 by Miss R. Cwilich. It was a subcolony of the bugs tested by Cwilich & Mer.

Cimex hemipterus

Normally susceptible colony. This was kindly supplied in December 1957 by Dr A. Smith from an unsprayed region of Tanganyika (Kwasunga).

Hong Kong strain. A colony of bugs suspected of DDT-resistance was sent by the Urban Services Department, Hong Kong, in April 1954, and has since been maintained in culture.

Mogadiscio strain. In April 1957 two samples of bugs were sent from Somalia by Dr M. Maffi. Only one survived; this originated in Mogadiscio, " a practically unsprayed zone ". Nevertheless, it was found to be DDT-resistant.

Mombasa strain. In November 1957 a colony of bugs was sent from Mombasa by Mr L. Peverett. They were taken from an infestation where dieldrin treatments were observed to be failing.

Gambia strain. This was kindly supplied by Dr Bringam, Medical Officer of Health, Bathurst, where DDT had been found ineffective.

Results and Conclusions

The results of tests with DDT and certain other insecticides against various bug colonies, are set out in Table 4, and similar data for BHC and the cyclodiene compounds are given in Table 5. Median lethal concentrations for the normal colonies have been estimated graphically from probit/log-concentration regression lines, and are set out in Table 6.

Attempts to assess levels of relative resistance quantitatively are hampered by two difficulties. The cases of moderate resistance to the chlorinated residual insecticides do not give straight probit/log-concentration regression lines, owing to heterogeneity. Strains showing high levels of resistance, on the other hand, are almost completely immune; so that although LC_{50} values have been estimated in some cases by extrapolation, they can only represent crude approximations.

In spite of the limitations in quantitative assessment of resistance mentioned, there are several interesting conclusions which can be drawn from the data.

(1) The general resistance characteristics of the various strains can be shown as follows, " O " indicating susceptibility, "xx" moderate resistance and " xxx " high resistance:

Species	*Origin*	*Date collected*	*Resistance to*	
			DDT series	*BHC-dieldrin series*
C. lectularius	London	1937	0	0
"	Israel	1957	xxx	xxx
C. hemipterus	Tanganyika (Kwasunga)	1957	0	0
"	Mombasa	1957	xxx	xxx
"	Hong Kong	1954	xx	0
"	Somalia (Mogadiscio)	1957	xx	0
"	Gambia (Bathurst)	1958	xx	xxx

Taking into account the data on strains of *C. hemipterus* resistant to dieldrin but not to DDT of Hamon et al. (1957) and Smith,[1] it appears that bed-bugs can develop resistance either to DDT and analogues or to BHC and dieldrin analogues or to both groups simultaneously. The levels of resistance found in certain strains do not appear to be in accordance with the available information on field usage of insecticides; thus, bugs from Bathurst were resistant to BHC and dieldrin and those from Mogadiscio to DDT, despite no evidence of field use of the relevant insecticides. Similar discrepancies have previously been reported for houseflies in Nigeria (Busvine & Harrison, 1953), and Taiwan (Liu, 1958) and for *Culex fatigans* in Malaya

[1] In a report submitted in 1957 to the East African Institute of Malaria and Vector-borne Diseases.

TABLE 4. RESULTS OF TESTS WITH VARIOUS STRAINS OF BED-BUG AGAINST DDT, DDT ANALOGUES, AND SIMILAR INSECTICIDES

Insecticide *	Concentration (%)	Percentage kills						
		C. lectularius		*C. hemipterus*				
		Laboratory	Israel	Kwasunga	Hong Kong	Mombasa	Mogadiscio	Bathurst
DDT	4	100	4		58	7	53	69
	3	91			31		45	—
	2	79		100	28		46	55
	1	37		85	21		7	0
	0.5	13		55	10			
	0.25	—		33	—			
DDT + DMC (1:1)	3	71	10		68	5	55	
	1	26		83	25			
	0.5			65				
	0.25			20				
Methoxychlor	2	75	7	100	77	0	17	
	1	50		91	54			
	0.5			78				
	0.25			8				
Perthane	8		63		79			
	4	100	22		78			
	2	88			66			
	1	58			23			
	0.5	17						
Prolan	2	100	16		81			
	1	100			84			
	0.5	37			43			
Pyrethrins	0.4		81		91	47		
	0.2	100	64	100	78			
	0.1	86	29	95	63			
	0.03	30		10	8			
Lethane 384	4	100						
	2	91	78	88	90			
	1	59	26	44	66			
Average numbers of bed-bugs per test		38	25	17	34	21	21	23

* Perthane is 2,2-bis-(*p*-ethylphenyl)-1,1-dichlorothane.
Prolan is 1,1-bis-(*p*-chlorophenyl)-2-nitropropane.
Lethane 384 contains β-butoxy-β'-thiocyano-diethyl ether, the concentrations given being based on this active principle.

TABLE 5. RESULTS OF TESTS WITH VARIOUS STRAINS OF BED-BUG AGAINST BHC AND DIELDRIN ANALOGUES

Insecticide	Concentration (%)	Percentage kills						
		C. lectularius		*C. hemipterus*				
		Laboratory	Israel	Kwasunga	Hong Kong	Mombasa	Mogadiscio	Bathurst
γ-BHC	1		96			65		100
	0.5		54			47		86
	0.2		26			20	100	0
	0.1	97			100		59	
	0.03	25		100	95		13	
	0.01	5		52	41			
	0.003				17			
Dieldrin	4		22			4		0
	3		17					
	0.2						91	
	0.1	83					84	
	0.075	51					—	
	0.05	21		100	93		14	
	0.025	0		27	64			
	0.013			9	33			
Aldrin	20		20					
	10		6					
	0.25	87						
	0.10	38						
Endrin	4		60					
	2		18					
	1		0					
	0.2	100						
	0.1	65						
	0.05	40						
Isodrin	8		46					
	4		27					
	0.2	95						
	0.1	33						
	0.05	10						
α-chlordane	20		13					
	10		9					
	2	86						
	1	68						
	0.5	37						
β-chlordane	20		17					
	10		12					
	0.5	100						
	0.2	44						
	0.1	17						
Average numbers of bed-bugs per test		32	27	18	23	20	20	18

TABLE 6. COMPARATIVE RESISTANCE OF TWO STRAINS OF *C. LECTULARIUS* TO VARIOUS INSECTICIDES *

Strain	LC_{50} with DDT and analogues						
	DDT	DDT+ DMC	methoxy-chlor	Perthane	Prolan	pyrethrins	Lethane 387
Laboratory	1.2	1.8	1.0	0.9	.55	.04	0.9
Israel	⩾ 4	⩾ 3	⩾ 2	6.5	> 2	0.15	1.3

Strain	LC_{50} with γ-BHC, dieldrin and analogues						
	γ-BHC	dieldrin	aldrin	endrin	isodrin	α-chlordane	β-chlordane
Laboratory	0.04	0.07	0.12	0.07	0.11	0.65	0.25
Israel	0.45	(30)	(50)	3.5	(9)	(approx. 200)	(approx. 200)
Resistance of Israel strain	11	(400)	(400)	50	(80)	(300)	(800)

* Figures in parentheses are based on extrapolations.

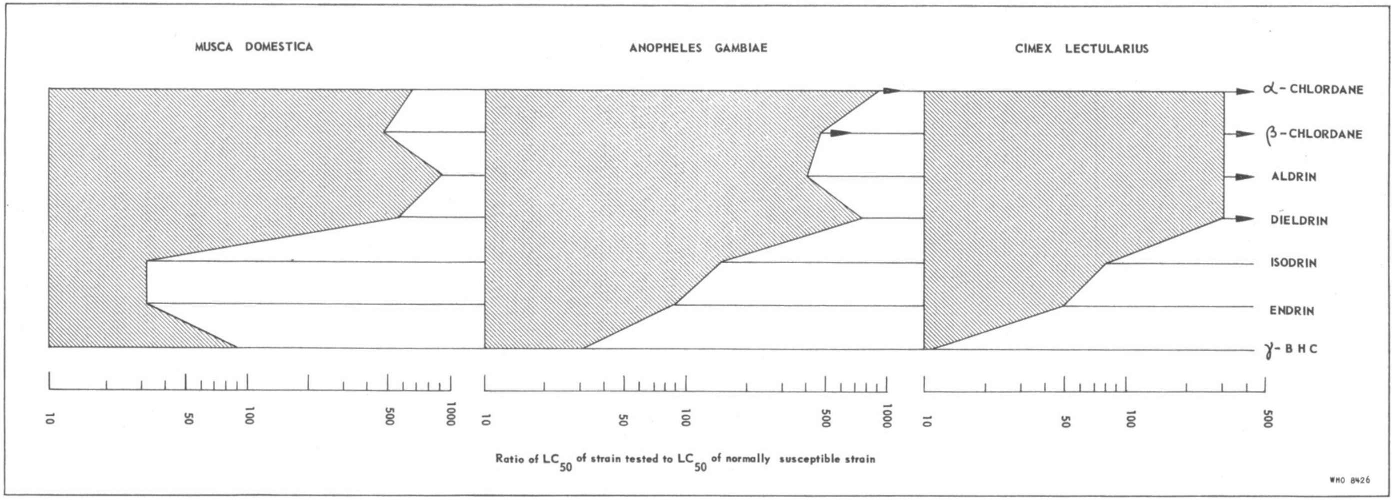
(Wharton, 1958). However, it would seem dangerous to assume the possibility of resistant populations existing before wide use of insecticides, in view of the difficulty of ascertaining exactly how much insecticide has been used in any particular tropical town.

(2) DDT-resistance in bugs involves resistance to methoxychlor, Prolan and Perthane and it is not overcome by addition of the " DDT synergist " DMC (1,1-bis-(*p*-chlorphenyl) ethanol). There is therefore no positive evidence in favour of a dehydrochlorination detoxification mechanism.

(3) There are definite indications of resistance to pyrethrins in strains resistant to DDT, reaching about 10 times normal in the Mombasa strain. Similar correlation of DDT-resistance with some degree of pyrethrin resistance has been previously observed with houseflies (Busvine, 1953). The absence of concomitant differences in susceptibility to Lethane 384 suggests that this is not mere vigour tolerance.

(4) The toxicological relations of various bug strains to the γ-BHC and dieldrin series of insecticides are consistent with those of other insects showing resistance to this group of poisons. Resistance to γ-BHC induces tolerances of dieldrin and *vice versa*; also involved are aldrin, endrin, isodrin, α- and β-chlordane. Regardless of which insecticide develops the

RESISTANCE SPECTRA OF STRAINS OF THREE INSECTS WHICH HAVE DEVELOPED RESISTANCE TO BHC, DIELDRIN AND ANALOGUES



Arrows indicate unknown values greater than the point shown.

The origins of the resistant strains are: *M. domestica* from Uruguay (Busvine, 1954); *A. gambiae* from Nigeria (Davidson, 1958); *C. lectularius* from Israel (this paper).

resistance, higher tolerance levels develop to dieldrin and chlordane than to γ-BHC. This has been observed with *Musca domestica* in several countries (Busvine, 1954), with *Blattella germanica* in the USA (Keller et al., 1956) with *Anopheles gambiae* in Nigeria (Davidson, 1958) and with *Culex fatigans* in Malaya (Wharton, 1958).

The approximate resistance levels of the Israel bug strain to the BHC-dieldrin group of poisons have been estimated (Table 6) and the resulting resistance spectrum is compared with corresponding ones for *Musca domestica* and *Anopheles gambiae* in the accompanying figure. The general similarity, especially between the bed-bug and the mosquito, is quite striking and suggests that a common defence mechanism against these insecticides may be developed in many different kinds of insect.

RÉSUMÉ

On a signalé en 1948 déjà la résistance au DDT des punaises de lit. Elle a été depuis lors maintes fois confirmée. L'auteur a repris la question expérimentalement, selon une méthode simple, qu'il décrit. Deux souches de *Cimex lectularius* et cinq de *C. hemipterus* — la plupart ayant présenté une résistance aux insecticides sur le terrain — ont été soumises à l'action des insecticides 5 jours après avoir été nourries, et durant 5 jours, période au bout de laquelle la mortalité semble se stabiliser. Malgré diverses difficultés dans les calculs statistiques dues en particulier à l'hétérogénéité du matériel, l'auteur a pu tirer certaines conclusions.

Les punaises peuvent présenter une résistance soit au DDT et à ses analogues, ou au HCH et à la dieldrine, soit aux deux groupes simultanément. Cette résistance ne paraît pas toujours liée à l'usage des insecticides sur le terrain (le même fait a été signalé chez les mouches et chez *C. fatigans* en Malaisie). Les données dont on dispose ne permettent toutefois pas d'affirmer l'existence de populations résistantes avant le traitement par les insecticides. La résistance au DDT ne paraît pas en relation avec un processus de détoxification par déchloruration. Elle s'accompagne d'une résistance aux pyréthrines (déjà observée chez les mouches). La résistance au HCH et à la série de la dieldrine offre le même caractère que chez d'autres insectes (mouches, blattes, *A. gambiae*, *C. fatigans*). Une étude comparative des spectres de résistance d'une souche de *C. lectularius* et de souches de *Musca domestica* et d'*A. gambiae*, indique qu'un même mécanisme de défense doit exister chez diverses espèces d'insectes.

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